

AVIDITY MEASUREMENTS ON ANTI-LPS ANTIBODIES INDUCED DURING SALMONELLA INFECTIONS IN PIGS

WIUFF, C., HEEGAARD, P. and LIND, P.¹

Traditional serological tests provide only a semiquantitative measure of antibodies present but not their binding strength to the test antigen. We have established an enzyme immunoassay (ELISA) for avidity (functional affinity) measurements, based on the ability of urea to dissociate low avidity antibody-antigen complexes. In the avidity ELISA LPS-coated microtiter plates are incubated with pig sera and afterwards treated with urea. In parallel the same sera are incubated without subsequent urea treatment. The ratio between the OD-values from incubations with and without urea treatment gives the avidity index (Hedman et al. 1989, Kallio-Kokko et al. 1992, Lappalainen et al. 1992). Antibodies of low avidity are dissociated easily and give a low index, whereas antibodies of high avidity are unaffected by the treatment resulting in high indices (Hedman et al. 1989). We have applied this method to investigate the development of avidity for IgG and IgM antibodies during experimental *Salmonella* Typhimurium infection in pigs. Avidity assays are of special interest in follow-up studies on herd level, where information is needed regarding the onset of infection or regarding the presence of chronically infected animals.

METHODS

ELISA procedure : LPS purified from formalin killed cultures of *S. Typhimurium* (no. 3389-1/92, O:1, 4, 5, 12) was used as coating antigen. PolySorp (Nunc, Denmark) microtitre plates were coated with 0.75 µg/ml LPS in 0.1 M sodium carbonate, 1.0 M NaCl, pH 9.6 overnight at 4°C. The plates were then blocked with incubation buffer (PBS, 0.05% Tween 20, 1% BSA) for 15 min, and washed once with washing buffer (PBS, 0.05 % Tween 20). Positive Typhimurium sera from experimentally infected pigs were diluted in incubation buffer 1:400 or 1:100, titrated 2-fold in 8 steps in duplicate, and incubated for 1 h at room temperature. All plates were made in duplicates. After incubation with sera half of the plates were treated for 3 x 5 min. with 8 M urea, PBS, 0.05 % Tween 20 with agitation, and the urea was washed away 3 times with washing buffer. The other half of the plates were washed 3 x 5 min in washing buffer. This was followed by incubation for 1 h with peroxidase-conjugated rabbit anti Ig (P164, DAKO, Denmark) diluted 1:2000 in incubation buffer at room temperature, or by incubation with peroxidase-conjugated goat anti-swine IgG(γ) (Kirkegaard & Perry, Maryland, USA) diluted 1:2500 or peroxidase-conjugated rabbit anti-swine IgM (μ) (Bethyl, Texas, USA) diluted 1:6000, followed by 3 times washing. Finally, 100 µl substrate (0.01 % H₂O₂-1,2-orthophenylenediamine dihydrochloride in 0.1 M citrate, pH 5) was added to each well and incubated for 10 min. The reaction was stopped with 100 µl H₂SO₄ and the optical density (OD) was read at 490 nm - 650 nm for background correction.

¹ Danish Veterinary Laboratory, 27 Bülowsvej, 1790 Copenhagen V, Denmark

Estimation of avidity index : For each dilution of the sera the avidity index (A.I. _x) is calculated. The avidity index (A.I.) is defined as the average of all A.I. _x complying with $OD_{+urea} < OD_{-urea}$, in which OD is defined as $OD = OD_{serum} - OD_{blind}$, can be used.

$$\text{Avidity index}_{\text{dilution } x} : A.I. _x = \frac{OD_{\text{(dilution } x) + \text{urea}}}{OD_{\text{(dilution } x) - \text{urea}}}$$

$$\text{Avidity index} : A.I. = \left(\sum_{i=1}^n A.I. _x \right) / n$$

RESULTS

When testing different experimental conditions, it appeared that avidity indices could not be estimated for sera with low OD ($OD < 0.3$). Increasing the urea concentration from 6 M to 8 M and the urea incubation times combined with agitation, resulted in lower avidity indices. By increasing the strength of the urea treatment, antigen-antibody bonds with low affinity are more easily broken. Using the rabbit antisera to pig Ig (P164) gave higher avidity indices than seen when using goat anti swine IgG, probably reflecting a higher affinity between the peroxidase conjugate and the pig sera. Avidity indices for IgM were in general lower and more uniform than for IgG, despite the possible multivalent binding of IgM to the antigen.

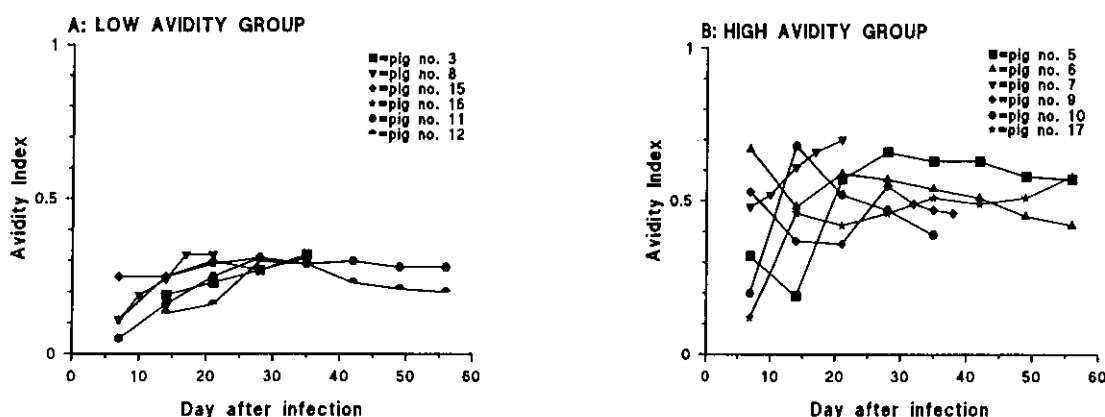


Fig. 1 Development of IgG avidity index after an experimental infection of pigs with *Salmonella* Typhimurium at day 0. A) low avidity group, B) high avidity group.

The development of IgG avidity of 12 pig sera obtained after experimental *Salmonella* Typhimurium infection is shown in fig. 1. The majority of the sera had very low avidity indices (< 0.3) 7 days after the infection, which is the first day of bleeding after sero conversion. Avidity indices generally increased forward to day 21, whereafter values tended to stabilize. The sera could be divided into two groups, a low avidity group stabilized at a level with values under 0.3

(fig. 1A) and a high avidity group stabilized at a level between 0.4 and 0.7 (fig. 1B). Furthermore the IgM/IgG ratios were measured in parallel with the IgG avidity measurements. At day 7, nearly all the sera had very high IgM titers and very low IgG titers. Pig 6 and 9, which had high avidities at day 7, had also much higher IgG titers at day 7 than the other sera. This could implicate that IgM in the other samples simply were blocking for access of the IgG to the LPS.

DISCUSSION

A method for estimating the avidity of antibodies in salmonella positive sera towards LPS has been established. From the initial experiments it can be concluded that the avidity increases until 21 days after the infection and then stabilizes either at low level ($OD < 0.3$) or at a higher level ($OD > 0.4$).

Since the immune response towards LPS is mainly T-cell independent and probably does not involve antigen processing and MHC presentation, it is very interesting to study the avidity development of these antibodies directed towards a carbohydrate epitope as LPS. In virus infections involving protein antigens, the avidity index can be used for distinguishing between newly infected and chronically infected individuals (Hedman et al. 1989, Kallio-Kokko et al. 1992, Lappalainen et al. 1992). The same development of avidity in anti-LPS antibodies in salmonella infected pigs was found in the present study, although the time scale was different. The development of avidities of both IgG, IgM and IgA over a time course will be further studied, and sought applied in a similar way as for virus infections, as an improvement of the serodiagnosis of salmonella infected pigs.

REFERENCES

- Hedman, K. and Rousseau, S.A., 1989. Measurements of Avidity of Specific IgG for Verification of Recent Primary Rubella. *Journal of Medical Virology*, 27: 288-292.
- Kallio-Kokko H., Valpalathi, O., Hedman, K., Brummer-Korvenkontio, M., and Vaheri, A., 1992. Puumala Virus Antibody and Immunoglobulin G Avidity Assays Based on a Recombinant Nucleocapsid Antigen. *Journal of Clinical Microbiology*, 31 (3): 677-680.
- Lappalainen, M., Koskela, P., Koskiniemi, M., Ämmälä, P., Hiilesmaa, V., Teramo, K., Raivio, K.O., Remington, J. and Hedman, K., 1992. Toxoplasmosis Acquired during Pregnancy: Improved Serodiagnosis Based on Avidity of IgG. *The Journal of Infectious Diseases*, 16: 691-697.